Final Research Report

[redacted]

CHRONIC TOXICITY OF [redacted]
TO *Danio rerio* IN AN EARLY-LIFE STAGE TOXICITY
TEST UNDER FLOW-THROUGH CONDITIONS

[redacted]

ABSTRACT

The purpose of this study was to assess the toxicity of the test substance dissolved in fresh water, on the early life stages of *Danio rerio*, in a 36-day flow-through test complying with the OECD Guideline No. 210, 17 July 1992.
The test criterion of toxicity used was the effects on hatching, larvae mortality, morphological abnormalities and growth of *Danio rerio* exposed to the test substance over the test period.

The nominal concentrations used in the study were as follows:
0, 0.75, 1.5, 3.0, 6.0 and 12 mg/L

Analytical determinations of the test solutions were made on 20 occasions during the test. The concentrations were found to remain stable to within 20% of the nominals. The nominal concentrations were used to calculate the effect concentrations.

The validity criteria were respected:

- the dissolved oxygen concentration was between 60 and 100% of the air saturation value throughout the test,
- water temperature remained between 23 and 27°C over the test period and did not differ more than ±1.5°C between successive days
- The post-hatch success (until the end of the test) was greater than 70% in the control.

The No Observed Effect Concentration (NOEC) is determined as the concentration used in the study that is immediately below the Lowest Observed Effect Concentration (LOEC), the latter derived statistically from the data using the appropriate statistical test.

Pre-hatch mortality was found in all concentrations, but was not as high as in the control and was therefore not concentration related.
Post-hatch survival showed a concentration related effect and therefore an EC_{10} was determined of 8.6 mg/L. The LOEC was considered to be 12 mg/L and the NOEC therefore 6.0 mg/L.
No teratogenic malformations were noted for any larvae at any concentration.
Length data were statistically assessed using multi-comparison tests. The LOEC was found to be 12 mg/L and the NOEC therefore 6.0 mg/L.

Based on results from length, the Lowest Observed Effect Concentration (LOEC) was considered to be 12 mg/L and the No Observed Effect Concentration (NOEC) was determined 6.0 mg/L.

CHRONIC TOXICITY OF [redacted] TO *DANIO RERIO* IN EARLY LIFE STAGE TOXICITY TEST UNDER FLOW-THROUGH CONDITIONS

Sponsor [redacted]

Study monitor

STUDY ORGANISATION

Location [redacted]

Study director

Quality Assurance Unit

Management

Initiation date of the study
Completion date of the study

ARCHIVING AND STORAGE

The project file including the final report, amendments to the final report, the study plan, amendments to the study plan, records of quality assurance inspections, all letters, memos and notes and raw data pertaining to the study will be retained in the archives of [redacted] for a period of ten years. Other records including master schedule sheet, laboratory notebooks, logbooks, records of the maintenance and calibration of equipment, summary of training, curricula vitae and job descriptions of the personnel involved in the study, records related to location and storage of the test substance will also be kept in the [redacted]rchives for a period of ten years. Test material will be stored deep frozen under the sample code T 07024 for ten years or only as long as the quality of the test substance permits evaluation.

GLP COMPLIANCE STATEMENT

The study reported here was carried out according to the study plan in compliance with the OECD Principles of Good Laboratory Practice excluding test substance data submitted by the sponsor. The report contains an accurate description of the results.

[redacted]

[redacted]

voedsel en waren autoriteit

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 29-31 January 2008 at

[redacted]

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Physical-chemical testing, environmental toxicity studies on aquatic and terrestrial organisms and tests on behaviour in water, soil and air; bioaccumulation.

NEDERLAND

Den Haag, 19 March 2008

Dr Th. Helder
Manager GLP Compliance Monitoring Program

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QUALITY ASSURANCE STATEMENT

This report was audited by the Quality Assurance Unit contracted by [redacted]. It is considered to be an accurate presentation of the methods and procedures applied in the course of the study and an accurate reproduction of the data recorded.
Listed below are the dates of inspection of this study by the Quality Assurance Unit and the dates on which its findings were reported to Study Director and Management.

Dates of inspection	Phase of the study	Dates of reporting
29-10-2007	Study plan	29-10-2007
08-11-2007	Egg selection and distribution, test substance stock preparation	08-11-2007
14-11-2007	Test conditions; analysis	14-11-2007
23-11-2007	Day 15: analytical data	25-11-2007
16-02-2008	Finalizing: pH, O_2, conductivity measurements, killing, counting and measuring fish	16-02-2008
	Final report	

[redacted]

CONTENTS

INTRODUCTION

1.1 Objectives

The objective of this study was to assess the lethal and sub-lethal effects of sodium chlorate on hatching and early-life stages (embryo, larvae and juveniles) of *Danio rerio*, in a flow-through test.

1.2 Principle of the test

Fish eggs were exposed in two groups to the test substance added to test medium at a range of concentrations. Under otherwise identical test conditions the effects on hatching, larvae mortality, morphological abnormalities and growth of *Danio rerio* exposed to the test substance is recorded over a period of approximately 35 days.

1.3 Regulatory compliance

The study will be conducted in compliance with the following Good Laboratory Practice regulations:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

2. TEST GUIDELINES, MODIFICATIONS AND DEVIATIONS

The study was carried out in accordance with OECD Guidelines for testing of chemicals no. 210 (9.1) without modification of the test guideline.

3. MATERIALS

3.1 Test substance

The test substance (project sample code T 07024) was supplied by the sponsor. Data on the handling, stability, composition, purity or other characteristics of the test substance supplied by the sponsor was accepted and used without further verification.

- Product name [redacted] (also known as [redacted])
- chemical name [redacted]
- CAS reg. no. [redacted]

Dimethylphthalate 131-11-3

- appearance Transparent slightly viscous liquid
- water solubility [redacted]
- vapour pressure Expected to be negligible
- batch number BOE 07059
- stability Stable when kept in a closed bottle in the refrigerator and away from light
- storage until required refrigerator / at room temperature away from light

An analytical certificate provided by the sponsor is presented in annex 1.

3.2 Chemicals

All reagents used will be of reagent grade quality and obtained from J.T. Baker Chemicals BV, Deventer, The Netherlands and Acros, Tilburg, The Netherlands or Fluka Chemie GmbH, CH-947 Buchs, Switzerland.

3.3 Test vessels

Monoblock glass aquaria with a holding capacity of 1.5 litres in an open flow-through circuit driven by peristaltic/syringe pumps.

3.4 De-ionised water

The de-ionised water used in the study contained less than 10 µg/l of copper, had a conductivity of less than 5 µS/cm and less than 2.0 mg/L NPOC-content.

3.5 Test room, temperature control and light regime

The test was carried out in a temperature-controlled room. The test temperature was between 24.4 and 26.2°C and the actual temperature was kept constant within ±1.5°C between successive days.

The light regime was set at 16 h of ambient light per day, provided by fluorescent tubes.

3.6 Apparatus

The dissolved oxygen concentrations were determined electrochemically using an oxygen electrode and meter. The pH was determined with a pH meter. The temperature was measured with a thermocouple and recorder and a digital thermometer.
The flow-through system consists of multi-head Gilson Watson Marlow peristaltic pumps set in parallel followed by *in situ* mixing of the stock solution to achieve appropriate dilutions.

3.7 Test medium

Test water: Dutch Standard Water (DSW) was used for the study. A known quantity of the demineralised water passed directly into a reservoir tank and the appropriate hardness was obtained by adding salts to water. The pH of the resulting solution was between 6.0 and 8.5 (generally in the range of 8.0 ± 0.5) and conductivity measured at the beginning of the test was 561µs/cm, which is in the range of 550 and 650 µs/cm given for DSW. Water hardness is measured monthly in the production tank to verify that it meets the criteria. During the test it was measured in the DSW tank on day 11 and found to be 13.1 °dH (equal to 234 mg $CaCO_3$/L) which is within the accepted range of 140 to 250 mg $CaCO_3$/L.

3.8 Test animals

• Species	Zebra fish (*Danio rerio*)
• Justification of this choice	the zebra fish is a fish species recommended in the OECD guideline and is generally accepted by regulatory authorities for this type of study.
• Origin	Broodstock: Dierenspeciaalzaak Engelen, Rijnstraat 17, 6811 EW Arnhem, The Netherlands. The broodstock are maintained in Akzo Nobel Environmental Chemistry laboratory.
•	Eggs, Akzo Nobel Environmental Chemistry laboratory. Fertilized fish eggs collected in the laboratory were used to start the test as soon as possible after laying. The developmental phase of the eggs at test initiation was between zygote and blastodisc cleavage stage equivalent to about 45 minutes after spawning under laboratory conditions.
• Acclimatization	no acclimatization period as the test should start as soon as possible after the eggs have been fertilized.
• Allocation to study	fertilized eggs were selected using a pipette or a hand-net, without preference, and randomly assigned to test vessels.
• Number of eggs	at least 80 fertilized eggs per concentration divided into two replicates of at least 40 eggs each.

4. METHODS

4.1 Test solutions

Preparation of the stock solutions

To prepare the stock solutions, 1.00 ± 0.008 g of test substance were weighed then dissolved directly in 10 litre DSW (see § 3.7). Previous non-GLP studies on stability have revealed that the test substance is stable for up to 48 h in DSW. The obtained preparations were agitated mechanically for between 1 and 24 hours in an attempt to completely dissolve the test substance (previous non-GLP studies have shown that an aqueous solution of 100 mg/L of test substance in DSW can be obtained within one hour by mechanical agitation). Stock solutions of this batch of peroxide at 100 mg/L have been shown to be stable in DSW for periods up to 3 days.

The pH of the stock solution(s) were checked and found to be between 8.0 and 8.3 and were not adjusted.

Preparation of the test solutions

Test solutions were prepared by further dilution of the stock solution with DSW.

A geometric series of concentration was used. The ratio between two consecutive concentrations did not exceed 2.

Test vessels (aquaria) were filled using a flow-through system from the test solution containers immediately after preparation.

The pH of the test solutions were between 7.6 and 8.1 and close to the value of test water (± 0.5 units).

The test concentrations to be used in the test are as follows: 0, 0.75, 1.5, 3.0, 6.0 and 12 mg/L.

4.2 Test conditions

The test vessels were checked for visible residual food and faeces each day and these were removed to avoid accumulation of waste and the risk of bacterial contamination.

The test conditions were set as follows:

- Duration of test — the study was stopped 30 days after the end of hatching in the control.
- Loading — maximum 1 g of biomass (eggs, embryos, larvae or juvenile fish) per litre of test solution per 24 hours and not exceeding 2 g per litre of test solution at any time.
- pH — not adjusted during the test.
- Oxygen concentration — dissolved oxygen maintained at or above 60% of the air saturation value at that temperature.

Temperature was measured in all aquariums at the beginning of the test, once a week thereafter and at the end of the test. The temperature in one of the test vessels was also monitored continuously.
Dissolved oxygen and pH values were measured in one vessel per concentration at the beginning of the test once a week thereafter and at the end of the test.
Conductivity was measured in one vessel of the control and the highest test concentration where animals were still alive at the start of the test, once a week thereafter and at the end of the test.

4.3 Sampling

As the test substance is known to be unstable under the conditions of the study, samples were taken three times per week and pooled between replicates. Samples were filtered over a Pall 0.45 pm GHP Acrodisc filter, transferred into 10 ml HPLC vials and analyzed immediately. When considered necessary by the SD, further samples were taken within 24 hours, as described above, and analyzed immediately.

4.4 Feeding

Larvae were fed at the free-swimming stage (approximately 4 days after hatch) with protozoa from an infusorium containing mainly *Paramecia* species. The protozoa were captured and pipetted into an appropriate quantity of DSW before being bottled and included in the test set up as food for the fish. Feeding started on day 5 of the test. The first day of feeding the larvae received 3 times 10 ml, on day 6 to 14 of the test 4 times 10 ml was given every day. On day 15 to 17 the larvae received again 3 feedings per day. Ten ml of food was automatically transferred using a peristaltic pump, into the test vessels until the fish were old enough to accept brine shrimp nauplii.
On day 18 of the test the juveniles in the control were old enough to eat brine shrimp nauplii, but in the test substance concentrations some animals were too small to be able to eat the nauplii at this time, therefore all aquaria received 2 to 3 droplets of brine shrimp nauplii from a Pasteur pipette and 10 ml *Paramecia* per day until day 32 of the test. From day 33 until the end of the test only nauplii were fed.

4.5 Study design

At least 80 eggs per concentration were randomly selected and divided equally between two replicates of five concentrations and one control.
The study was started as soon as possible after fertilisation and as close as possible to blastodisc cleavage stage.
The eggs were suspended in the test solutions by placing them in baskets covered on the underside with plastic gauze with a mesh size small enough to prevent the eggs from falling through.
On the first day of the study, white, opaque (dead) eggs were counted in each replicate and removed. The number of surviving eggs was reduced randomly to approximately 30 in the control replicates and in the replicates containing the test substance. Once the living eggs hatched the baskets were removed.

When swim-up began in the control, surviving larvae were thinned randomly so that the number of fish in each replicate at each concentration was identical (minimum of 20 larvae per replicate).

4.6 Observations

In all test vessels, the initial stage of embryo development at the start of study was recorded. Eggs, embryos, larvae and juveniles were checked for visible abnormalities (abnormal appearance and behaviour) and mortality each day.

Criteria for death were as follows:

- for eggs: particularly in the early stages, a marked loss of translucency and change in colouration, caused by coagulation and/or precipitation of protein, leading to a white opaque appearance,
- for embryos: absence of body movement and/or absence of heart-beat,
- for larvae and juvenile fish: immobility and/or absence of respiratory movement and/or absence of heart-beat and/or white opaque colouration of central nervous system and/or lack of reactions to mechanical stimulus.

Dead embryos, larvae and juvenile fish were removed as soon as observed.

The other criteria used were as follows:

- the time to start and end of hatch and number hatched,
- the length of all surviving fish to the nearest 0.1 mm at the end of the test,
- the individual weight of these fish (blotted wet weight), was not possible. So fish were divided into 2 groups per aquarium and weighed (to calculate the mean individual wet weight of the surviving animals per replicate) to the nearest 0.1 mg,
- the number of deformed larvae,
- the number of larvae behaving abnormally.

4.7 Analysis

The method used to determine the concentration of the test substance in the test medium is described in annex 3.

The mean concentrations measured were between 80 and 120% of the nominal concentrations, therefore nominal concentrations were used for subsequent calculations.

5. RESULTS

5.1 Preliminary test

No preliminary acute toxicity test was performed as sufficient data were available to provide a series of concentrations for use in the reproduction study (Migchielsen, 2002).

However, a full non-GLP study on test substance analysis, stability and recovery was performed and reported prior to the GLP study being initiated (Thomas, *et al.*, 2007).

5.2 Water quality

Temperature measurements in the test solutions: min. 24.4; max 26.2°C.

Constant record of temperature over test time: 24.0 to 25.35°C.

Oxygen concentration: min. 7.1; max. 8.4 mg O_2/L.

pH: min. 7.6; max. 8.1

Full results of Physico-chemical parameter measurements are presented in annex 2.

5.3 Analytical results

All test concentrations and the control were analysed. The test solutions were found to be stable over the test period. As concentrations were observed to be between 81.3 and 97.0 % of the nominals, all statistical evaluation has been based on nominal concentrations. A full description of the analytical method and results table is provided in annex 3. Table 1 gives the time weighted average of the tested concentrations during the test period. This was calculated by taking the mean of two consecutive sampling points and taking the time (i.e. number of days between the two samples) into account.

Example:

In a 36 day test the following measurements are done
0.63 mg/L is measured on day 0
0.67 mg/L on day 2
0.61 mg/L on day 5
....etc.
Time weighted average is ((0.63 + 0.67)/2 x 2/36) + ((0.67 + 0.61)/2 x 3/36) + ...etc.

Table 1: concentration of the time weighted average

Sample	Concentration (mg/L)	% of nominal
Control	< LOD	---
0.75 mg/L	0.65	87.3
1.5 mg/L	1.47	97.8
3.0 mg/L	2.62	87.3
6.0 mg/L	4.93	82.2
12 mg/L	9.75	81.3

As all concentrations had a time weighted average >80% of the nominal concentration, the nominal was used thereafter to calculate the endpoints.

5.4 Data evaluation

The following data are presented in annex 4 for each concentration:

- cumulative mortality at embryo, larval and juvenile stages,
- time of start of hatching and end of hatching,
- incidence and description of morphological abnormalities, if any,
- incidence and description of behavioural effects, if any,

- length of surviving fish at the end of the study,
- numbers of healthy fish at the end of the test.

5.5 Hatching, animal mortality, physical or behavioural abnormalities and numbers of fish at the end of the study

First hatch was observed on day 3 of the study in the control and all test concentration. Hatching in the control was complete by day 6 and in the test concentrations by day 7. Not all fish (approximately 30) hatched at all concentrations.
In the control 23 coagulated eggs were counted in total. In all test concentrations the counted number was lower; 6 to 18 coagulated eggs (see annex 4). It was not always possible to make a proper count, even though the counts were made 6 days a week, the delicate nature of the biological material meant that decomposition occurs very quickly. It is possible that on the one day in the week they were not counted, some coagulated eggs were already decomposed and therefore not included in the final result. Because of this uncertainty no statistics were performed on number of living eggs per group. Despite this it can be concluded that no concentration related mortality occurred, the control having the greates count of coagulated eggs.

The numbers of surviving fish per concentration are given in annex 4. The numbers of fish present after swim-up in the control and thinning were reworked from the number alive at the end of the test and the dead fish counted during the test. This was done because it was not possible to count the living fish accurately and determine the exact number of fish after thinning.
One fish out of 50 died in the control during the test. In 0.75 mg/L 1 fish out of 53 died, in 1.5 mg/L no fish died, in 3.0 mg/L 4 fish out of 62 died, in 6.0 mg/L 2 fish out of 59 died and in 12 mg/L 17 fish out of 57 died during the test. Details are included in annex 4 and in the table below.

Table 2. Survival and percentage survival at the end of the test

Conc. (mg/L)	fish no. at the start of the test		survival		percentage survival	
replicate	I	II	I	II	I	II
Control	24	26	24	25	100	96.2
0.75	32	21	32	20	100	95.2
1.5	28	26	28	26	100	100
3.0	26	36	24	34	93.3	94.4
6.0	27	32	26	31	96.3	96.9
12	27	30	21	19	77.8	63.3

The No Observed Effect Concentration (NOEC) for survival was derived as the first concentration below the LOEC value, where survival showed no significant difference to the control values, using William's test (9.6). An EC_{10} was determined by maximum likelihood regression using the probit transformation. Confidence limits were computed on the basis of Fieller's theorem (9.7). All computations on survival were performed using the TOXCALC™ version 5.0 program.

For the calculations percentage survival was used, because the number of fish in each replicate at the start of the test were not all equal.

The LOEC is was determined as 12 mg/L and the NOEC is therefore 6.0 mg/L based on nominal concentrations. The EC_{10} for survival is 8.6 mg/L with 95% confidence limits of 7.0 to 9.6 mg/L.

No morphological or behavioural abnormalities were noted at any concentration at any time during the study.

5.6 Length and weight of fish at the end of the study

Details of length of fish are included in annex 4.
In the control the minimum and maximum size of fish were 4 and 14 mm respectively with an average length of 9.1 mm.
At 12 mg/L the minimum and maximum size of fish were 4 and 12 mm respectively with an average length of 8.3 mm.

Table 3. Average fish lengths (mm) with (standard deviations) at each concentration (mg/L)

Control	0.75	1.5	3.0	6.0	12.0
9.1 (1.8)	8.6 (1.4)	8.0 (1.2)	8.3 (1.3)	8.6 (1.5)	8.3 (2.2)

Length data were found to be not normally distributed using Chi-square test for normality, therefore a transformation was done. The square root of the length was taken and then the data were found to be normally distributed and an analysis of variance was performed. The data passed Hartley's test for homogeneity of variance. Analysis of variance was performed on length data using the Bonferroni t-test (annex 5) this could not be verified with the Dunnett's test, because this test needs equal sample size.

Multi-comparison tests of group length animals were employed. Significant differences from the control were found for 1.5, 3.0 and 12 mg/L. 6.0 mg/L did not differ significantly from the control. The statistical results of 1.5 and 3.0 mg/L do not seem to be concentration related because in 3.0 mg/L the fish have a higher average length than in 1.5 mg/L. When Bonferroni- and Dunnett's tests are performed on the average length per replicate (see annex 5) no significant differences are found at any of the test concentrations compared to the control. Therefore the results of 1.5 and 3.0 mg/L are not used and the LOEC is considered to be 12 mg/L. The NOEC therefore is 6.0 mg/L.

Weighing of the fish was carried out. The fish were blotted dry and wet weight was measured. During the weighing process it was noticed that loss of water occurred, this led to significant variations in weight and therefore these results could not be used.

Based on results from length, the Lowest Observed Effect Concentration (LOEC) is 12 mg/L and the No Observed Effect Concentration (NOEC) is 6.0 mg/L.

5.7 Any other biological effects observed

No other biological effects were observed during the study.

6. CONCLUSION

The No Observed Effect Concentration (NOEC) is determined as the concentration used in the study that is immediately below the Lowest Observed Effect Concentration (LOEC), the latter derived statistically from the data using the appropriate statistical test.

Pre-hatch mortality was found in all concentrations, but was not as high as in the control and was therefore not concentration related. The NOEC for this endpoint is ≥ 12 mg/L.

Post-hatch survival showed a concentration related effect and an EC_{10} of 8.6 mg/L was determined. Using ANOVA a LOEC of 12 mg/L was calculated and the NOEC is therefore 6.0 mg/L for this endpoint.

No teratogenic malformations were noted for any larvae at any concentration.

Length data were statistically assessed using multi-comparison tests. The LOEC was found to be 12 mg/L and the NOEC is therefore 6.0 mg/L.

Based on the overall results from pre-hatch mortality, post-hatch survival and length, the Lowest Observed Effect Concentration (LOEC) was considered to be 12 mg/L and the No Observed Effect Concentration (NOEC) was determined as 6.0 mg/L.

7. DEVIATIONS FROM THE STUDY PLAN

- pH was measured in one vessel of the control and all concentrations where animals were still alive at the start of the test, once a week thereafter and at the end of the test rather than at just the highest concentration where animals are still alive at the start of the test, as stated in the study plan.
- The stock solutions were stirred between 1 and 24 hours instead of 2 to 4 hours. As the test substance was measured regularly during the study and found to be consistently within the expected range, this deviation is not considered to have had an impact on the study
- On day 18 of the test the juveniles in the control were old enough to eat brine shrimp nauplii, but in the test concentrations some animals were too small to be able to eat the nauplii, therefore all aquaria received brine shrimp nauplii and 10 ml *Paramecia* per day until day 32 of

the test instead of feeding them only brine shrimp nauplii. From day 33 until the end of the test only nauplii were fed.

- Weight was not used as an endpoint due to inaccuracy of weighing results. As length measurements of individual fish are expected to be directly related to weight parameter, this deviation is not considered to have an impact on the scientific integrity of the study.

8. QUALITY CRITERIA

- The dissolved oxygen concentration was between 60 and 100% of the air saturation value throughout the test,
- Water temperature remained between 23 and 27°C over the test period and did not differ more than ±1.5°C between successive days
- The post-hatch success (until the end of the test) was greater than 70% in the control.
- The mean concentrations measured were between 80 and 120% of the nominal concentrations.

9. REFERENCES

9.1 OECD, (1992) Guidelines for testing of chemicals no. 210 Organisation for Economic Co-operation and Development, Paris.

9.2 Thomas P.C., Vos A., van Dam J., Kean M. & Helming B. (2007). Final interim report Development and validation of an analytical method for Butanox P50 and development and optimization of the flow through test set up for chronic daphnia and early life stage fish tests. Prelim study for T 07024 ODC & OFE.

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9.6 William's D.A., 1972. The comparison of seven dose levels with a zero-dose control. Biometrics 28, pp. 519-531.

9.7 Zerbe G.O., 1978. On Fieller's theorem and general linear model. The American Statistician, Vol. 32, 3, pp. 103-105.

[redacted]

ANNEX 1

CERTIFICATE OF ANALYSIS

[redacted]

Certificate of Analysis

[redacted]

Product name	: -
Chemical name	: [redacted]
Batch number	: [redacted]

Test results:

Method	Analysis of	[redacted]	[redacted]
Jo/72.10, Jo/72.11, Jo/02.1	Peroxidic compounds (sum) *See page 2 for a specification*	[redacted]	[redacted]
HPLC	[redacted]	[redacted]0)	
HPLC	[redacted]	% m/m	[redacted]
Amp/88.9	[redacted]	% m/m	[redacted]

*1 bracketed values are estimated 95% confidence intervals

Archive code : [redacted]

Analytical documentation : [redacted]

Authorized by

Name : [redacted]

Date : [redacted]

Signature : [redacted]

[redacted]

[redacted]

[redacted]

Certificate of Analysis

[redacted]

[redacted]

specification of the peroxidic compounds

structure	% m/m
[redacted]	

[redacted]

ANNEX 2

PHYSICO-CHEMICAL PARAMETER MEASUREMENTS

Table 1: **pH-values**

Nominal Test Conc. [mg/L]	0 Hours	7 Days	14 Days I	21 Days	28 Days	End of test
Control	7.9	7.9	7.8	7.7	7.8	7.9
0.75	8.0	8.0	7.8	7.7	7.8	8.0
1.5	8.1	8.1	7.8	7.6	7.8	7.9
3.0	8.1	8.1	7.8	7.7	7.9	8.0
6.0	8.1	8.1	7.8	7.7	7.8	8.1
12.0	8.1	8.1	7.8	7.7	7.8	8.0

Table 2: **Conductivity (us/cm)**

Nominal Test Conc. [mg/L]	0 Hours	7 Days	14 Days I	21 Days	28 Days	End of test
Control	561	647	620	606	574	612
12.0	564	647	628	606	566	608

Table 3: **Temperature (°C)**

Nominal Test Conc. [mg/L]	0 Hours	7 Days	14 Days I	21 Days	28 Days	End of test
Control	24.9	25.1	25.4	24.9	25.5	26.0
0.75	25.2	24.8	25.0	24.8	25.3	26.2
1.5	25.3	25.2	25.3	24.7	25.1	25.1
3.0	24.8	25.3	25.1	24.9	25.2	24.9
6.0	24.5	24.8	25.4	25.1	24.8	25.3
12.0	25.1	24.6	24.8	24.8	24.4	25.4

Table 4: **Dissolved Oxygen Concentration (mg/L)**

Nominal Test Conc. [mg/L]	0 Hours	7 Days	14 Days I	21 Days	28 Days	End of test
Control	8.1	7.8	8.1	7.6	7.6	7.8
0.75	8.4	7.9	8.1	7.6	7.7	7.5
1.5	8.2	7.8	7.7	7.6	7.3	7.2
3.0	8.2	7.8	7.9	7.4	7.8	8.0
6.0	8.1	7.7	7.9	7.2	7.4	7.8
12.0	8.2	7.7	7.7	7.4	7.1	7.4

ANNEX 3

Description of the analytical procedure for the quantification of [redacted] using a HPLC system

1. Introduction

A method is described to determine the concentration of [redacted]n water. Procedures and instrumentation are based on High Performance Liquid Chromatography combined with on-line Solid Phase Extraction and UV detection. Analysis is based on two peaks, i.e. MIPKP Type 3 and MIPKP Type 4, representing the active ingredient of the test substance. The concentration of the test substance in the analytical method is calculated as the sum of these 2 peaks. Samples were quantified using a calibration curve.

2. Analytical procedure

The following conditions were found to be suitable for the determination of the test compound for concentrations of 0.5 to 100 mg/L in de-ionised water, Dutch Standard Water and M4 medium.

- Autosampler: Spark, model Triathlon
- Pump: Knauer Smartline 1000
- Gradient manager: Knauer Smartline 5000
- Mobile phase:

0 min.	30% A	70% B
5 min.	30% A	70% B
15 min.	100% A	0% B
17 min.	100% A	0% B
18 min.	30% A	70% B
20 min.	30% A	70% B

A= Acetonitrile B= HPLC water

- Column: Waters Symmetry 4,6 x 150mm 5µm C18 RP column, with guard column.
- On-line SPE cartridge: PLRP-s 15-25µm
- Flow rate: 1.0 ml/min
- Detector: UV/VIS detector
- Wavelength: 220 nm
- Injection volume: 8 ml (trapped on SPE cartridge in 4 min. with flow of 2 ml/min.)
- Integrator: VG Chromatography server
- Integration software: Atlas 2002R1

For preparing the standards at the beginning of the test period a stock solution of test substance in de-ionized water was made. For the calibration series dilutions in de-ionized water in a concentration

range of 0 – 100 mg/L (n ≥ 5) from the stock solution were made. The samples taken during the test were quantified using this calibration series.

During the test period every week a fresh stock solution of test substance was prepared. Before every analysis series a control sample from the middle range of the calibration standards, prepared from the stock solution of test substance of the current week, was analyzed. This control standard was analyzed at the beginning of every sample series and at a minimum rate of one per ten samples and at least at the end of each sample series.

3 Calculation of concentrations

Quantification was done by measurement of peak areas. The concentrations of the test substance in the samples were calculated from the relation between concentration of standards (Cs) and peak area (PAs) obtained with linear regression analysis:

PAs = Cs * Slope + constant

$$\text{Sample concentration} = \frac{\text{Sample peak area} - \text{constant}}{\text{slope}}$$

As peak area of the test substance the sum of the peak areas from the two components, MIPKP Type3 and MIPKP Type 4, was considered.

4. Reproducibility and validation

With the system described above the two components, considered to represent the test substance, eluted after about 15 minutes.

The analytical method was found to be linear over the concentration range of 0.5 to 100 mg/L of the test substance, using the conditions described above. Every separate HPLC calibration series should give a linear regression with a squared regression coefficient r^2 ≥ 95% (n>=5). Control standards analyzed during the analyses should be within 10% of the expected values based on the calibration curve. If this was not the case a second control standard was analysed. If this standard still showed a deviation of ≥10% of the expected value, the calibration procedure was repeated.

Table 1: Calibration standards of the test substance

calibration sample	Concentration (mg/L)	Peak Area (µVs)
ST 0	0.00	0
ST 0.5	0.50	12.078
ST 1.0	1.00	24.587
ST 2.0	2.00	50.606
ST 5.0	5.00	128.709
ST 10	10.00	260.396
ST 20	20.00	531.618

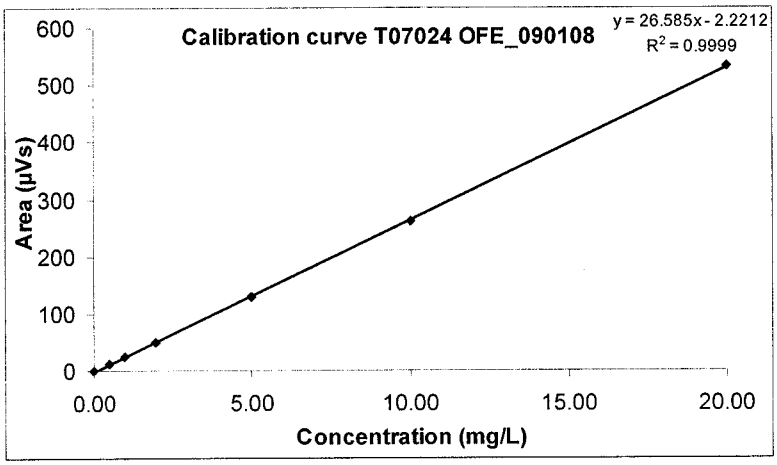

Figure 1: calibration curve of the test substance in deionised water

Table 2: Measured concentration of pooled samples per test concentration in mg/L

day	Control	0.75 mg/L	1.5 mg/L	3.0 mg/L	6.0 mg/L	12 mg/L
0	< LOD	0.70	1.80	3.06	5.51	10.02
3	< LOD	0.70	0.99	3.05	5.34	9.28
4			1.74			10.53
5	< LOD	0.69	1.57	2.88	5.14	10.25
7	< LOD	0.75	1.78	2.74	5.21	10.22
10	< LOD	0.44	1.17	2.04	4.44	9.19
11	< LOD	0.75	1.67	2.48	4.82	9.93
12	< LOD	0.64	1.51	2.65	4.75	9.47
14	< LOD	0.63	1.23	2.63	5.12	9.86
17	< LOD	0.55	1.34	2.53	4.76	8.85
19	< LOD	0.70	1.42	2.77	5.11	8.53
20						9.50
21	< LOD	0.73	1.43	2.41	4.66	8.56
24	< LOD	0.59	1.31	2.23	4.31	8.61
26	< LOD	0.73	1.60	2.82	4.82	10.62
28	< LOD	0.67	1.40	2.55	4.54	10.33
31	< LOD	0.56	1.56	2.58	4.75	10.31
33	< LOD	0.58	1.67	2.81	5.23	10.51
34		0.69				
36	< LOD	0.81	1.62	2.25	5.69	11.18

LOD= Limit of detection

Limit of Detection and Quantification

$$\text{Limit of detection} = \frac{3 * \text{standard error of calibration curve}}{\text{slope from the calibration curve}}$$

$$\text{Limit of quantification} = \frac{10 * \text{standard error of calibration curve}}{\text{slope from the calibration curve}}$$

LOD and LOQ were calculated and found to be 0.123 and 0.409 mg/l, respectively.

ANNEX 4

Fish mortality, length and weight data
0 mg/L

Date (2008)	11.01	12.01 W	13.01 W	14.01*	15.01	16.01	17.01	18.01	19.01 W	20.01 W	21.01	22.01	23.01	24.01	25.01	26.01 W	27.01 W	28.01	29.01
Replicate No.	Number of fish																		
1								24	24	24	24	24	24	24	24	24	24	24	24
2								25	25	25	25	25	25	25	25	25	25	25	25
Mortality [N] 1																			
∑ Mortality [%] 1																			
Mortality [N] 2							1												
∑ Mortality [%] 2							3.8												
Immobile [N]																			
Coagulated eggs [N]		7	7	20	20	23													

Date (2008)	30.01	31.01	01.02	02.02 W	03.02 W	04.02	05.02	06.02	07.02	08.02	09.02 W	10.02 W	11.02	12.02	13.02	14.02	15.02	Total
Replicate No.	Number of fish																	
1	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
2	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Mortality [N] 1																		0
∑ Mortality [%] 1																		0
Mortality [N] 2																		1
∑ Mortality [%] 2																		3.8
Immobile [N]																		0
Coagulated eggs [N]																		23

W: Weekend
* Eggs thinned to approx. 30
Shaded column: Hatch complete

0.75 mg/L

Date (2008)	11.01	12.01 W	13.01 W	14.01*	15.01	16.01	17.01	18.01	19.01 W	20.01 W	21.01	22.01	23.01	24.01	25.01	26.01 W	27.01 W	28.01	29.01
Replicate No.	Number of fish																		
1								32	32	32	32	32	32	32	32	32	32	32	32
2								21	21	21	21	21	21	21	21	21	21	20	20
Mortality [N] 1																			
∑ Mortality [%] 1																			
Mortality [N] 2																		1	
∑ Mortality [%] 2																		4.8	
Immobile [N]																			
Coagulated eggs [N]		9	9	18	18	18													

Date (2008)	30.01	31.01	01.02	02.02 W	03.02 W	04.02	05.02	06.02	07.02	08.02	09.02 W	10.02 W	11.02	12.02	13.02	14.02	15.02	Total
Replicate No.	Number of fish																	
1	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
2	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Mortality [N] 1																		0
∑ Mortality [%] 1																		0
Mortality [N] 2																		1
∑ Mortality [%] 2																		4.8
Immobile [N]																		0
Coagulated eggs [N]																		18

W: Weekend

* Eggs thinned to approx. 30

Shaded column: hatch complete

1.5 mg/L

Date (2008)	11. 01	12. 01 W	13. 01 W	14. 01*	15. 01	16. 01	17. 01	18. 01#	19. 01 W	20. 01 W	21. 01	22. 01	23. 01	24. 01	25. 01	26. 01 W	27. 01 W	28. 01	29. 01
Replicate No.	Number of fish																		
1								28	28	28	28	28	28	28	28	28	28	28	28
2								26	26	26	26	26	26	26	26	26	26	26	26
Mortality [N] 1																			
∑ Mortality [%] 1																			
Mortality [N] 2																			
∑ Mortality [%] 2																			
Immobile [N]																			
Coagulated eggs [N]		10	10	13	13	13													

Date (2008)	30. 01	31. 01	01. 02	02. 02 W	03. 02 W	04. 02	05. 02	06. 02	07. 02	08. 02	09. 02 W	10. 02 W	11. 02	12. 02	13. 02	14. 02	15. 02	Total
Replicate No.	Number of fish																	
1	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
2	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
Mortality [N] 1																		0
∑ Mortality [%] 1																		0
Mortality [N] 2																		0
∑ Mortality [%] 2																		0
Immobile [N]																		0
Coagulated eggs [N]																		13

W: Weekend
* Eggs thinned to approx. 30
# Fish thinned
Shaded column: hatch complete

3.0 mg/L

Date (2008)	11.01	12.01 W	13.01 W	14.01*	15.01	16.01	17.01	18.01#	19.01 W	20.01 W	21.01	22.01	23.01	24.01	25.01	26.01 W	27.01 W	28.01	29.01
Replicate No.	Number of fish																		
1								26	26	26	26	26	26	26	26	26	25	24	24
2								36	36	36	36	36	36	36	36	36	36	34	34
Mortality [N] 1																	1	1	
∑ Mortality [%] 1																	3.8	7.7	
Mortality [N] 2																		2	
∑ Mortality [%] 2																		5.6	
Immobile [N]																			
Coagulated eggs [N]		6	6	6	6	6													

Date (2008)	30.01	31.01	01.02	02.02 W	03.02 W	04.02	05.02	06.02	07.02	08.02	09.02 W	10.02 W	11.02	12.02	13.02	14.02	15.02	Total
Replicate No.	Number of fish																	
1	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
2	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34
Mortality [N] 1																		2
∑ Mortality [%] 1																		7.7
Mortality [N] 2																		2
∑ Mortality [%] 2																		5.6
Immobile [N]																		0
Coagulated eggs [N]																		6

W: Weekend
* Eggs thinned to approx. 30
# Fish thinned
Shaded column: hatch complete

6.0 mg/L

Date (2008)	11.01	12.01 W	13.01 W	14.01*	15.01	16.01	17.01	18.01#	19.01 W	20.01 W	21.01	22.01	23.01	24.01	25.01	26.01 W	27.01 W	28.01	29.01
Replicate No.	Number of fish																		
1								27	27	27	27	26	26	26	26	26	26	26	26
2								32	32	32	32	32	32	32	32	32	32	32	32
Mortality [N] 1												1							
∑ Mortality [%] 1												3.7							
Mortality [N] 2																			
∑ Mortality [%] 2																			
Immobile [N]																			
Coagulated eggs [N]		11	11	12	13	14													

Date (2008)	30.01	31.01	01.02	02.02 W	03.02 W	04.02	05.02	06.02	07.02	08.02	09.02 W	10.02 W	11.02	12.02	13.02	14.02	15.02	Total
Replicate No.	Number of fish																	
1	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
2	32	32	32	32	32	31	31	31	31	31	31	31	31	31	31	31	31	31
Mortality [N] 1																		1
∑ Mortality [%] 1																		3.7
Mortality [N] 2						1												1
∑ Mortality [%] 2						3.1												3.1
Immobile [N]																		0
Coagulated eggs [N]																		14

W: Weekend
* Eggs thinned to approx. 30
# Fish thinned
Shaded column: hatch complete

12 mg/L

Date (2008)	11.01	12.01 W	13.01 W	14.01*	15.01	16.01	17.01	18.01#	19.01 W	20.01 W	21.01	22.01	23.01	24.01	25.01	26.01 W	27.01 W	28.01	29.01
Replicate No.	Number of fish																		
1								27	27	27	27	27	25	25	25	25	25	23	22
2								30	30	30	30	30	30	30	30	30	30	29	27
Mortality [N] 1													2					2	1
∑ Mortality [%] 1													7.4					14.8	18.5
Mortality [N] 2																		1	2
∑ Mortality [%] 2																		3.3	10
Immobile [N]													1			0			
Coagulated eggs [N]		2	2	2	7	7													

Date (2008)	30.01	31.01	01.02	02.02 W	03.02 W	04.02	05.02	06.02	07.02	08.02	09.02 W	10.02 W	11.02	12.02	13.02	14.02	15.02	Total
Replicate No.	Number of fish																	
1	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
2	25	24	23	22	21	21	21	21	21	21	21	21	19	19	19	19	19	19
Mortality [N] 1	1																	6
∑ Mortality [%] 1	22.2																	22.2
Mortality [N] 2	2	1	1	1	1								2					11
∑ Mortality [%] 2	16.7	20	23.3	26.7	30								36.7					36.7
Immobile [N]																		1
Coagulated eggs [N]																		7

W: Weekend
* Eggs thinned to approx. 30
# Fish thinned
Shaded column: hatch complete

[redacted]

INDIVIDUAL BODY LENGTH MEASUREMENTS (MM) INCLUDING CAUDAL FIN

	Concentration (mg/L)					
	0		0.75		1.5	
Fish no.	Vessel I	Vessel II	Vessel I	Vessel II	Vessel I	Vessel II
1	11	11	9	11	7	8
2	7	8	9	9	7	8
3	10	9	8	9	8	8
4	8	12	7	11	7	8
5	10	10	8	9	9	7
6	9	9	9	9	7	8
7	11	8	6	9	8	8
8	7	7	9	8	8	8
9	9	14	8	9	8	8
10	10	11	10	8	8	8
11	10	9	8	10	10	9
12	7	10	11	11	8	9
13	7	11	8	8	8	12
14	8	8	8	9	10	5
15	7	7	8	10	10	5
16	8	8	8	5	10	6
17	10	11	7	11	9	6
18	11	9	8	9	8	7
19	9	8	10	9	8	7
20	9	9	9	8	8	8
21	10	4	6		9	8
22	8	10	9		7	9
23	7	12	7		7	8
24	10	11	9		6	9
25	9		7		7	9
26			7		8	9
27			6		8	
28			9		7	
29			9			
30						
31						
32						
33						
34						
MEAN	8.9	9.4	8.2	9.1	8.0	7.9

	Concentration (mg/L)					
	3.0		6.0		12	
Fish no.	Vessel I	Vessel II	Vessel I	Vessel II	Vessel I	Vessel II
1	10	6	9	8	10	11
2	8	7	9	7	9	9
3	8	8	9	8	9	5
4	9	8	10	9	9	8
5	6	7	9	8	9	8
6	7	9	9	7	11	8
7	10	8	7	9	5	10
8	8	10	9	12	11	10
9	9	10	7	8	10	6
10	9	6	10	7	9	7
11	10	6	9	9	9	10
12	8	8	12	9	11	6
13	7	10	6	9	10	4
14	6	8	8	11	12	5
15	10	7	9	8	9	8
16	10	7	10	9	7	7
17	8	8	9	9	8	5
18	9	9	6	7	11	5
19	9	6	8	7	9	4
20	8	8	12	7	9	
21	10	7	6	9	10	
22	9	9	9	9		
23	8	9	8	10		
24	9	8	10	10		
25		6	10	9		
26		8	6	10		
27		10		9		
28		9		8		
29		11		6		
30		9		9		
31		8		9		
32		10				
33		8				
34		9				
Mean	8.5	8.1	8.7	8.6	9.4	7.2

ANNEX 5

STATISTICAL RESULTS

Results of fish length
All fish lengths taken separately

SQUARE ROOT(Y)

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	20.569	74.294	117.274	74.294	20.569
OBSERVED	29	53	130	76	19

Calculated Chi-Square goodness of fit test statistic = 11.0988
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Transform: SQUARE ROOT(Y)

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 3.21
Closest, conservative, Table H statistic = 3.6 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 30
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 50.17
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Transform: SQUARE ROOT(Y)

Bartletts test for homogeneity of variance

Calculated B statistic = 27.77
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 50.17
Used for Chi-square table value ==> df (#groups-1) = 5

[redacted]

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.152	0.230	3.108
Within (Error)	301	22.252	0.074	
Total	306	23.404		

Critical F value = 2.29 (0.05,5,120)
Since F > Critical F REJECT Ho:All groups equal

Transform: SQUARE ROOT(Y)

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	3.009	9.143		
2	0.75 mg/l	2.915	8.551	1.720	
3	1.5 mg/l	2.813	7.963	3.648	*
4	3.0 mg/l	2.874	8.310	2.564	*
5	6.0 mg/l	2.928	8.632	1.537	
6	12 mg/l	2.859	8.325	2.598	*

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=120,5)

Transform: SQUARE ROOT(Y)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	49			
2	0.75 mg/l	49	0.763	8.3	0.592
3	1.5 mg/l	54	0.746	8.2	1.180
4	3.0 mg/l	58	0.734	8.0	0.833
5	6.0 mg/l	57	0.736	8.1	0.511
6	12 mg/l	40	0.804	8.8	0.818

Average fish length per replicate

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 3.040

W = 0.947

Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 8.02
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

[redacted]

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.700	0.340	0.671
Within (Error)	6	3.040	0.507	
Total	11	4.740		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	9.150	9.150		
2	0.75 mg/l	8.650	8.650	0.702	
3	1.5 mg/l	7.950	7.950	1.685	
4	3.0 mg/l	8.300	8.300	1.194	
5	6.0 mg/l	8.650	8.650	0.702	
6	12 mg/l	8.300	8.300	1.194	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.75 mg/l	2	2.015	22.0	0.500
3	1.5 mg/l	2	2.015	22.0	1.200
4	3.0 mg/l	2	2.015	22.0	0.850
5	6.0 mg/l	2	2.015	22.0	0.500
6	12 mg/l	2	2.015	22.0	0.850

Transform: NO TRANSFORMATION

ANOVA TABLE

[redacted]

SOURCE	DF	SS	MS	F
Between	5	1.700	0.340	0.671
Within (Error)	6	3.040	0.507	
Total	11	4.740		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	9.150	9.150		
2	0.75 mg/l	8.650	8.650	0.702	
3	1.5 mg/l	7.950	7.950	1.685	
4	3.0 mg/l	8.300	8.300	1.194	
5	6.0 mg/l	8.650	8.650	0.702	
6	12 mg/l	8.300	8.300	1.194	

Bonferroni T table value = 3.14 (1 Tailed Value, P=0.05, df=6,5)

Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.75 mg/l	2	2.238	24.5	0.500
3	1.5 mg/l	2	2.238	24.5	1.200
4	3.0 mg/l	2	2.238	24.5	0.850
5	6.0 mg/l	2	2.238	24.5	0.500
6	12 mg/l	2	2.238	24.5	0.850

[redacted]

FIRST AMENDMENT TO THE FINAL REPORT

Chronic toxicity of MIPKP in DMP to *Danio rerio* in an Early-Life Stage toxicity test under flow-through conditions

Study code

[redacted]

AMENDMENT

On page 8 in paragraph 1.1 "sodium chlorate" should be changed into "[redacted] in DMP".

REASON FOR AMENDMENT

On page 8 in paragraph 1.1 sodium chlorate is erroneously mentioned as the test substance.

APPROVAL

[redacted]

[redacted]